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WASHINGTON, D.C. 20460

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM:

June 28, 2004

Subject: EPA Id No.: 058001. Azinphos methyl: Review of the developmental toxicity study (2002, MRID No.: 45711201).

DP Barcode No.: D284275
Submission No.: S618584
PC Code No.: 058001
TXR No.: 0050916

From: John Doherty 6/28/04
Developmental Neurotoxicity Review Committee
Health Effects Division 7509C

To: Margaret Rice
Special Review and ReRegistration Division 7507C

Through: Susan Makris 7/6/04
Chairperson
Developmental Neurotoxicity Review Committee
Health Effects Division 7509C

Background, Conclusions and Comments:

The Developmental Neurotoxicity Review Committee (DNTRC) has completed its review of the developmental neurotoxicity study (2002, MRID No.: 45711201) with azinphos methyl and has determined that the study is classified as UNACCEPTABLE/Guideline. The study does not meet current criteria for an acceptable guideline developmental toxicity study (870.6300) for an organophosphate insecticide because there was no evidence that the pups actually were exposed to the test material during either gestation or lactation. In particular, there was no inhibition of cholinesterase in the pups to indicate that the pups were actually exposed. The data-call-in for organophosphates insecticides specifically requires that the study demonstrate that the pups were exposed during lactation. A copy of the DER for this study is attached. The study is further identified in the following table.

Table. Identification of Study Reviewed and Executive Summary.

Study	Executive Summary
870.6300. Developmental neurotoxicity study-rats Bayer Corporation, Study No.: 01-D72-C1, June 20, 2002, MRID No.: 45711201.	In a developmental neurotoxicity study (2002, MRID 45711201) azinphos-methyl (89.6% a.i., Lot/batch #: 903-0098) was administered to 30 female Wistar Hannover Crl:WI (Glx/BRL/Han) IGS BR rats/dose, continuously in the diet, at dose levels of 0, 3, 10, or 15 ppm from gestation day (GD) 0 through lactation day (LD) 21. These doses corresponded to 0, 0.2, 0.7 or 1.1 mg/kg/day during gestation and 0.4 to 0.6, 1.4 to 2.1 or 2.0 to 3.9 mg/kg.day during lactation for maternal intake. No information was provided as to the actual intake of the pups during lactation. On post-natal day (PND) 4, litters were standardized to 8 pups/litter. Pups were weaned on post-natal day 22, after which time all animals received untreated diet. F_1 pups were assigned to subgroups in order to evaluate brain weights, neuropathology, learning and memory, motor activity, and acoustic startle response. *Maternal toxicity*. Clinical signs, body weights, body weight gains, food consumption, FOB, and reproductive performance were unaffected by treatment. **The maternal systemic LOAEL was not observed. The maternal systemic NOAEL is > 15 ppm (3.2 mg/kg/day) the highest dose during lactation.** ***Offspring toxicity.*** Offspring clinical signs, viability, body weights, sexual maturation, cholinesterase activity, FOB, motor activity, acoustic startle response, passive avoidance, watermaze, brain weights, morphometric measurements, gross pathology, and histopathology were unaffected by treatment. **The offspring LOAEL was not observed. The offspring NOAEL is > 15 ppm (up to 3.2 mg/kg/day) based on maternal consumption but not actual pup intake.** Cholinesterase inhibition. Decreases ($p<=0.05$) in plasma (23-43%), erythrocyte (54-63%), and brain (19-48%) cholinesterase were noted in the 10 and 15 ppm dose group dams when assessed at day 21 of lactation. Plasma, erythrocyte, and brain cholinesterase activities were comparable between control and treated pups on PND 4 and PND 21. **The plasma, erythrocyte, and brain cholinesterase LOAEL is 10 ppm (1.4 to 2.1 mg/kg/day) based on inhibition in the dams assessed on day 21 of lactation. The NOAEL is 3 ppm (0.4 to 0.6 mg/kg/day) based on intake during lactation.** This study is classified **UNACCEPTABLE/Guideline** and **does not** satisfy the guideline requirement (OPPTS 870.6300; OECD 426) for a developmental neurotoxicity study in rats. The limiting factor is that there was no demonstration that the pups were actually exposed to the azinphos methyl during either *in utero* or lactation. The purpose of the study is to determine if pups actually exposed to the test material have developmental effects. Therefore the study must demonstrate preferably by some reaction in the pups that the pups were actually exposed to the test material in order to meet current guideline criteria for an acceptable study for an organophosphate inhibitor.

(2)

DATA EVALUATION RECORD

AZINPHOS-METHYL

Study Type: §83-6, Developmental Neurotoxicity Study in Rats

Work Assignment No. 4-02-195 MRID 45711201

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer
Kelley VanVreede, M.S.

Signature: David McEwen for Kelley Van Vreede
Date: 9/3/02

Secondary Reviewer
David McEwen, B.S.

Signature: David McEwen
Date: 9/3/02

Program Manager
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 9/3/02

Quality Assurance
Steve Brecher, Ph.D.

Signature: Steve Brecher
Date: 9/3/02

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: John Doherty
Toxicology Branch, Health Effects Division (7509C)
EPA Work Assignment Manager: Pv Shah
Toxicology Branch, Health Effects Division (7509C)

Signature: [signature]
Date 6/15/04
Signature: [signature]
Date 6/15/04
Template version 11/01

TXR#: 0050916

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 058001 **DP BARCODE:** D284275
SUBMISSION NO.: S618584

TEST MATERIAL (PURITY): Azinphos-methyl (89.6% a.i.)

SYNONYMS: O,O-Dimethyl S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl] phosphorodithioate

CITATION: Sheets, L.P. and S.G. Lake (2002) A developmental neurotoxicity screening study with technical grade azinphos-methyl (Guthion®) in Wistar rats. Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS. Laboratory Study No.: 01-D72-CI, June 20, 2002. MRID 45711201. Unpublished.

SPONSOR: Bayer Corporation, Agriculture Division, Box 4913, Hawthorne Road, Kansas City, MO

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (2002, MRID 45711201) azinphos-methyl (89.6% a.i., Lot/batch #: 903-0098) was administered to 30 female Wistar Hannover Crl:WI (Glx/BRL/Han) IGS BR rats/dose, continuously in the diet, at dose levels of 0, 3, 10, or 15 ppm from gestation day (GD) 0 through lactation day (LD) 21. These doses corresponded to 0, 0.2, 0.7 or 1.1 mg/kg/day during gestation and 0.4 to 0.6, 1.4 to 2.1 or 2.0 to 3.9 mg/kg.day during lactation for maternal intake. No information was provided as to the actual intake of the pups during lactation. On post-natal day (PND) 4, litters were standardized to 8 pups/litter. Pups were weaned on post-natal day 22, after which time all animals received untreated diet. F_1 pups were assigned to subgroups in order to evaluate brain weights, neuropathology, learning and memory, motor activity, and acoustic startle response.

Maternal toxicity. Clinical signs, body weights, body weight gains, food consumption, FOB, and reproductive performance were unaffected by treatment. **The maternal systemic LOAEL was not observed. The maternal systemic NOAEL is > 15 ppm (3.2 mg/kg/day) the highest dose during lactation.**

Offspring toxicity. Offspring clinical signs, viability, body weights, sexual maturation, cholinesterase activity, FOB, motor activity, acoustic startle response, passive avoidance, watermaze, brain weights, morphometric measurements, gross pathology, and histopathology were unaffected by treatment. **The offspring LOAEL was not observed. The offspring**

NOAEL is > 15 ppm (up to 3.2 mg/kg/day) based on maternal consumption but not actual pup intake.

Cholinesterase inhibition. Decreases ($p<=0.05$) in plasma (23-43%), erythrocyte (54-63%), and brain (19-48%) cholinesterase were noted in the 10 and 15 ppm dose group dams when assessed at day 21 of lactation. Plasma, erythrocyte, and brain cholinesterase activities were comparable between control and treated pups on PND 4 and PND 21. **The plasma, erythrocyte, and brain cholinesterase LOAEL is 10 ppm (1.4 to 2.1 mg/kg/day) based on inhibition in the dams assessed on day 21 of lactation. The NOAEL is 3 ppm (0.4 to 0.6 mg/kg/day) based on intake during lactation.**

This study is classified **UNACCEPTABLE/Guideline** and **does not** satisfy the guideline requirement (OPPTS 870.6300; OECD 426) for a developmental neurotoxicity study in rats. The limiting factor is that there was no demonstration that the pups were actually exposed to the azinphos methyl during either *in utero* or lactation. The purpose of the study is to determine if pups actually exposed to the test material have developmental effects. Therefore the study must demonstrate preferably by some reaction in the pups that the pups were actually exposed to the test material in order to meet current guideline criteria for an acceptable study for an organophosphate inhibitor.

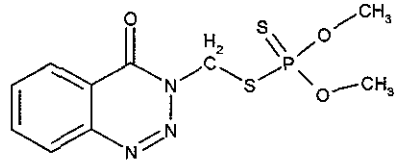
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Azinphos-methyl

Description:	Yellow solid
Lot/Batch #:	903-0098
Purity:	89.6% a.i.
Compound Stability:	The test substance was stable in the diet for 7 days at room temperature and 14 days frozen.
CAS # of TGAI:	86-50-0



2. Vehicle and/or positive control: Diet. No simultaneous positive control as included in the study. References to positive control studies were provided.

3. Test animals (P):

Species:	Rat	
Strain:	Wistar Hannover Crl:WI (Glx/BRL/Han) IGS BR	
Age at study initiation:	12 weeks (females)	
Wt. at study initiation:	205.0-213.6 g (females)	
Source:	Charles River Laboratories, Raleigh, NC	
Housing:	Individually, in stainless steel wire-bottomed cages except during mating, gestation, and lactation. During gestation and lactation, individual dams and their litters were housed in plastic cages.	
Diet:	Rodent Lab Chow 5002 (Purina Mills), *ad libitum* except during neurobehavioral testing.	
Water:	Tap water, *ad libitum* except during neurobehavioral testing	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	30-70%
	Air changes:	Not reported
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	At least 6 days	

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start: 1/8/2001 End: 4/12/2001

2. Study schedule: The test substance was administered to the maternal animals from gestation day (GD) 0 through lactation day (LD) 21. Pups were weaned on postnatal day (PND) 22, after which time all animals received untreated diet. F1 pups were assigned to subgroups in order to evaluate brain weights, neuropathology, learning and memory, motor activity, and acoustic startle response.

3. Mating procedure: Females were paired 1:1 with males of the same strain and source for a maximum of four consecutive days. Each female was examined daily during the mating period

to identify sperm cells in a vaginal smear or the presence of a copulatory plug. The day that sperm or a plug was found was designated gestation day (GD) 0, and each female was housed individually in a plastic nesting box.

4. Animal Assignment: Mated females were randomly assigned, stratified by body weight, to dose groups as indicated in Table 1. Offspring were assigned to testing subgroups at the time of litter standardization on post-natal day (PND) 4.

Table 1. Study design [a]

Experimental Parameter	Sub-group	Dose (ppm)			
		0	3	10	15
Maternal Animals					
No. of maternal animals assigned [b]	NA	30 for all groups			
FOB (GD 6 and 20)	NA	30 for all groups			
FOB (LD 11 and 21)	NA	10 for all groups			
Offspring					
Motor activity (PND 13, 17, 21, 60±2)	A	1 pup/sex/litter scheduled - 16 pups /sex actual.			
Acoustic startle habituation (PND 22, 38±2, 60±2)	B	1 pup/sex/litter scheduled -14 to 16 pups/sex actual.			
Passive avoidance (PND 22 and 29)	C	1 pup/sex/litter scheduled - 16 pups/sex actual			
Water maze (PND 60±2 and 7 days later)	C	1 pup/sex/litter scheduled - 16 pups/sex actual.			
FOB (PND 4, 11, 21, 35±1, 45±1, 60±2)	C	1 pup/sex/litter scheduled - 16 pups /sex actual			
Neuropathology and morphometric analysis (PND 21)	D	10/sex			
Cholinesterase determination (PND 21)	D	PND 4 pups had sexes combined. PND 21 has 5 - 10			
Brain weight (PND 75±5)	A, B, C	10/sex			
Omphthalmologic examination (PND 50-60)	A, B, C	10/sex			
Perfusion and neuropathology (PND 75±5)	A, B, C	Those selected for omphthalmologic examination			

a Data obtained from pages 28 and 29 of the study report.
b Approximate
NA Not applicable

5. Dose selection rationale: Dose levels were chosen based on the results of one-generation and two-generation reproduction studies in Wistar rats. Animals continuously received dietary concentrations of the test substance at 0, 5, 15 or 45 ppm, beginning approximately 13 weeks prior to mating. The only treatment-related finding noted at 5 ppm was decreased plasma and erythrocyte cholinesterase activities in maternal animals during lactation; cholinesterase inhibition (including brain) was noted also. Additionally at 15 ppm, maternal toxicity was characterized by reduced body weight and food consumption; offspring toxicity was characterized by decreased viability index and decreased body weight gains. At 45 ppm, mortality, moribundity, clinical signs of toxicity, and decreased body weight gains were observed in the dams; decreased viability index, reduced body weight gains, and decreased brain weights, and decreased brain cholinesterase were noted in the pups.

Based on the results of these reproduction studies, the doses presented in Table 1 were chosen for the developmental neurotoxicity study.

6. Dosage administration: All doses were administered to maternal animals continuously in the diet from GD 0 through LD 21.

7. Dosage preparation and analysis: Formulations were prepared weekly by mixing appropriate amounts of test substance with diet. Prepared diet mixtures were retained frozen until use. Homogeneity of the test substance in the diet was determined for the 3 and 15 ppm dose formulations prepared for study Week 1. Stability of the test substance in the diet (3 and 15 ppm formulations) was evaluated following 0, 1, 2, 3, 4, 5, and 7 days at room temperature and following 0, 7, and 14 days under freezer conditions. Concentration of the test substance in the diet was evaluated for the 3, 10, and 15 ppm test diets.

Results - Homogeneity Analysis (coefficient of variation):

3 ppm: 1.5%
15 ppm: 1.2%

Stability Analysis (range as % of day 0):

3 ppm: 92.3-98.5%
15 ppm: 95.0-102%

Concentration Analysis (range as % of nominal):

3 ppm: 87-100%
10 ppm: 91-97%
15 ppm: 92-97%

The analytical data indicated that the mixing procedure was adequate and the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals: Once daily checks for mortality, moribundity, and clinical signs were conducted for all maternal animals. In addition, detailed physical examinations were performed daily during treatment (GD 0 though LD 21).

An FOB (in the study report, this abbreviated FOB is referred to as "detailed observations") was conducted on GD 6 and GD 20 for all maternal animals. A minimum of 10 dams/dose were also examined on LD 11 and LD 21. This examination included "observations in the home cage, during handling, and outside the home cage in an open field, using standardized procedures"

FUNCTIONAL OBSERVATIONS (According to the tables on pages 104 to 127, the following parameters were investigated)	
X	Signs of autonomic function, including: 1) Lacrimation and salivation 2) Piloerection and exophthalmus, 3) Urination and defecation 4) Pupillary function 5) Palpebral closure
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors

Body weight and food consumption were measured weekly throughout treatment. In addition, dams were weighed on LD 4. Brain, erythrocyte, and plasma cholinesterase activities were measured in the dams (10/dose) on LD 21, using a modification of the Ellman method.

b. Offspring

1) Litter observations: The day of completion of parturition was designated as post-natal day (PND) 0. Live pups were counted, sexed (ano-genital distance) and weighed on PNDs 0, 4, 11, 17, and 21. At least once daily, offspring were examined cage-side for gross signs of mortality, morbidity, and clinical signs. Detailed clinical observations were made once daily before weaning and once weekly thereafter. Food consumption was measured weekly beginning the week of PND 28 (when pups were placed in individual housing). On PND 4, litters were standardized to 4 pups/sex//litter; excess pups were killed and discarded.

2) Developmental landmarks: Beginning on PND 38, all male offspring were examined daily for preputial separation. Beginning on PND 29, all female offspring were examined daily for vaginal patency. The age of onset was recorded. In addition, all pups were tested for the

presence of pupil constriction on PND 21.

3) **Postweaning observations:** Clinical observations were recorded daily for all animals. In addition, detailed clinical observations were recorded weekly during post-weaning. Body weights and food consumption were recorded weekly.

4) **Ophthalmology:** Animals that were selected for perfusion (minimum of 10/sex/dose) were subjected to ophthalmoscopic examinations at approximately 50-60 days of age. The eyes of each animal were examined with a slit lamp microscope and an indirect ophthalmoscope equipped with a condensing lens.

5) **Neurobehavioral evaluations**

i) **Functional observational battery (FOB-referred to as a detailed observational battery in the study report):** On PNDs 4, 11, 21, 35 (±1 day), 45 (±1 day), and 60 (±2 days), selected pups (approximately 16/sex/dose; subset C) were observed outside the home cage according to procedures outlined for the dams. In general, the neonates (PNDs 4 and 11) were not evaluated in the open field unless the observer considered it to be necessary.

ii) **Motor activity testing:** Activity was evaluated in 1 pup/sex/litter/exposure group (subset A) on PNDs 13, 17, 21, and 60 (±2 days). Motor and locomotor activity were measured by testing animals in figure eight mazes. Each test session was one hour in duration, and consisted of ten-minute intervals. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Habituation was evaluated as a decrement in activity over consecutive intervals of the test session.

iii) **Acoustic startle habituation:** Acoustic startle habituation testing was performed on 1 pup/sex/litter/exposure group (subset B) on PNDs 22, 38 (±2 days) and 60 (±2 days). The test session consisted of 50 trials that began following a 5 minute adaptation period at ambient noise levels. The rats were then presented with the startle-eliciting stimulus at 10 second intervals. The response amplitude was recorded and the baseline was subtracted.

iv) **Learning and memory testing:** Learning and memory testing was performed on 1 pup/sex/litter/exposure group (subset C). Passive avoidance testing was performed on PNDs 22 and 29; watermaze testing was performed on PND 60 (±2 days) and again seven days later. For both tests, only animals that demonstrated acquisition on the first day were tested for retention seven days later.

Passive avoidance test: After adaptation, individual animals were placed into the "lighted" compartment of a conditioning apparatus facing toward the light. After approximately 20 seconds, the trial began with the light being illuminated to signal the beginning of the trial and the door separating the two compartments opening, so that each rat was provided access to the non-illuminated side of the cage. When the rat crossed into the dark compartment, the door automatically closed, the shock was delivered, and the light switched off, signaling the end of the trial. At that time the animal was returned to the holding cage to await the next trial. If the rat

failed to cross within 180 seconds, it was returned to the holding cage and the latency assigned and arbitrary score of 180. The procedure was repeated until either the rat remained in the lighted compartment for 180 seconds on two consecutive trials or until 15 trials had elapsed, whichever occurred first. Rats that failed to meet the criterion during the learning phase were assigned a value of 15 for the trials-to-criterion variable. The test was repeated one week later. For the second trial, rats were placed in the illuminated side of the apparatus, given a 20 second acclimation period, and the latency to enter the dark side was recorded. Animals that either failed to reach criterion within 15 trials, or failed to cross during the first two trials during acquisition, were excluded from the retention phase of the experiment.

Water maze: On each test trial, the rat was placed into the starting position at the base of the M-maze stem, located between the two lateral arms. On the first trial (learning trial), the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the water and then removed from the maze. The initial arm chosen on this leaning trial was designated the incorrect goal during the subsequent 15 trials (maximum). Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal with the exit ramp and then removed from the water. Between trials, the animal was returned to a transport cage to wait for the next trial. The inter-trial interval was approximately 15 (±5) seconds. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was fifteen. Latency to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors during each trial. Animals that satisfied the above criteria within the 15 trial limit were tested for retention seven days following acquisition. Animals that failed to reach criterion during acquisition were excluded from the retention phase of the experiment.

6) **Cholinesterase determination:** Brain, erythrocyte, and plasma cholinesterase activities were measured in the offspring on PNDs 4 and 21 using a modification of the Ellman method. On PND 4, samples were collected from culled male and female pups, representing as many litters as possible. On PND 21, samples were collected from pups in subset D (5-10 pups/sex/dose). Animals were not fasted prior to blood collection.

7) **Pharmacokinetic data:** Pharmacokinetics were not evaluated in this study.

2. Postmortem observations

a. Maternal animals: Maternal animals were sacrificed by carbon dioxide asphyxiation on either GD 24 (rats that did not deliver) or LD 21 (following weaning). Gross necropsies were not performed.

b. Offspring: The offspring selected for perfusion on PND 21 (subset D) and at study termination (subsets A-C), as well as those selected for fresh brain weight determinations (approximately 10/sex/group from subsets A-C) were examined grossly.

Only the brain (with olfactory bulbs) was collected from the perfused animals on PND 21. Upon study termination, the brain and spinal cord, eyes (with optic nerves), selected peripheral nerves (sciatic, tibial, and sural), the gasserian ganglion, gastrocnemius muscle, and both forelimbs were collected. All tissues were fixed in 10% buffered formalin. The brain from each animal was weighed, sectioned, and examined microscopically. Additionally, the following (CHECKED X) tissues, to be examined microscopically, were collected from perfused animals at study termination:

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN	X	**SCIATIC NERVE**
X	Forebrain		Mid-thigh
	Center of cerebrum		Sciatic Notch
	Midbrain		
X	Cerebellum		
X	Pons		
	Medulla oblongata		
	SPINAL CORD		**OTHER**
X	Cervical swelling	X	Sural Nerve
X	Lumbar swelling	X	Tibial Nerve
X	Thoracic swelling		Peroneal Nerve
		X	Lumbar dorsal root ganglion
		X	Lumbar dorsal root fibers
	OTHER	X	Lumbar ventral root fibers
X	Gasserian Ganglion	X	Cervical dorsal root ganglion
	Trigeminal nerves	X	Cervical dorsal root fibers
X	Optic nerve	X	Cervical ventral root fibers
	Eyes		
X	Cauda equina		
	Gastrocnemius muscle		

Only tissues from the control and 15 ppm groups were subjected to microscopic examination and morphometric analysis. The following brain sections were measured: 1) frontal cortex thickness; 2) parietal cortex thickness; 3) caudate putamen horizontal width; 4) corpus callosum thickness; 5) hippocampal gyrus thickness; and 6) cerebellum height.

D. DATA ANALYSIS

1. Statistical analyses: In general, continuous data were initially assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed further using ANOVA, followed by Dunnett's test as necessary. Group means with unequal variances were analyzed using non-parametric procedures (Kruskal-Wallis ANOVA followed by the Mann-Whitney U test). The level of significance was set at $p \leq 0.05$, with the exception of Bartlett's test which was set at $p \leq 0.001$. The following data sets were analyzed by specific statistical procedures:

Parameter	Statistical test
Motor and locomotor total session activity data	ANOVA Dunnett's test
Interval motor and locomotor activity data	Repeated measures ANOVA (test interval and test occasion) ANOVA Dunnett's test
Acoustic startle response amplitude data, peak amplitude	ANOVA Dunnett's test
Acoustic startle response amplitude, block data	Repeated measures ANOVA (test block) Dunnett's test
Passive avoidance, latency data	Wilcoxon Test for time to failure
Passive avoidance, number of trials-to-criterion	Kruskal-Wallis and Wilcoxon tests for the acquisition phase Fisher's Exact Test for retention
Water maze, latency data	Univariate ANOVA Dunnett's test
Water maze, number of trials-to-criterion and number of errors	Kruskal-Wallis and Wilcoxon tests for the acquisition phase Fisher's Exact test for retention
Micropathology	Chi-Square One-tailed Fisher's Exact test

2. Indices

a. Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Mating index = # inseminated females/# females cohoused with males x 100

Fertility index = # pregnant females/# inseminated females x 100

Gestation index = # females with live pups/# pregnant females x 100

b. Offspring viability indices: The following viability (survival) indices were calculated from lactation records of litters in the study:

Live birth index = # live pups born per litter/total # pups per litter x 100

Viability index = # live pups on day 4 pre-culling per litter/# live pups born per litter x 100

Lactation index = # live pups on day 21 per litter/# live pups on day 4 post-culling per litter x 100

3. Positive control data: It was stated that previous studies (MRID 42770301 and Bayer report #109803) with untreated animals and rats treated with substances that increase (triadimefon) and decrease (chlorpromazine) motor activity have established the sensitivity, reliability, and validity of the test procedures. Additional studies (MRID 45441302) have been performed to establish test norms for the appropriate ages under these conditions and the effects of perinatal exposure to a reference chemical (methimazole) on activity in animals tested at these ages. However, no data were provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical and functional observations: No animals died during the study. Furthermore, no treatment-related clinical signs were noted in any dose group.

2. Body weight and food consumption: No treatment-related differences in body weights, body weight gains, or food consumption were noted during gestation or lactation (Table 2). Increased ($p \leq 0.05$) food consumption noted during LDs 7-14 in the 15 ppm dams (↑10%) was considered to be incidental.

TABLE 2. Mean (±SE) maternal body weight and food consumption. [a]

Observations	Dose (ppm)			
	Control	3	10	15
Gestation				
Mean body weight (g)				
Gestation day 0	205.0±2.83	213.6±2.61	207.6±2.54	210.7±2.70
Gestation day 6	225.8±2.99	232.3±2.71	225.6±3.10	227.6±2.76
Gestation day 13	249.7±3.68	258.1±3.02	254.0±2.90	256.0±2.64
Gestation day 20	313.1±4.62	319.2±4.23	320.1±4.01	319.4±4.64
Mean weight gain (g)				
Gestation days 0-20	107.9±3.47	105.6±2.93	112.5±2.03	108.8±3.32
Mean food consumption (g/animal/day)				
Gestation days 0-6	17.9±1.28	16.0±0.42	16.3±0.39	16.3±0.35
Gestation days 6-13	20.1±1.07	19.8±0.73	20.6±0.74	19.3±0.36
Gestation days 13-20	20.0±0.55	19.9±0.46	19.9±0.38	20.1±0.58
Lactation				
Mean body weight (g)				
Lactation day 0	239.6±4.69	247.6±3.51	244.9±3.24	245.8±3.17
Lactation day 4	256.9±4.36	264.7±3.48	261.9±3.57	258.9±4.17
Lactation day 7	265.3±4.30	272.9±3.00	256.6±5.15	267.3±3.51
Lactation day 14	275.1±5.73	292.9±3.53* (↑6)	283.6±3.85	286.8±2.99
Lactation day 21	266.7±6.57	283.6±2.93	279.7±3.54	268.6±6.60
Mean weight gain (g)				
Lactation days 0-21 [b]	27.1	36.0	34.8	22.8
Mean food consumption (g/animal/day)				
Lactation days 0-7	34.7±2.11	38.3±2.58	36.6±2.00	34.1±1.39
Lactation days 7-14	49.0±1.56	53.3±0.99	52.0±0.86	53.7±0.97* (↑10)
Lactation days 14-21	61.5±1.74	64.6±0.98	64.1±1.65	65.1±1.45

a Data obtained from Tables 3 through 4 and 6 through 7 on pages 62 through 65 and 69 through 72 in the study report; n = 19-30.

b Calculated by the reviewers.

* Statistically different from control, $p \leq 0.05$.

3. <u>Test substance intake</u>: Based on maternal food consumption, body weight and dietary analyses, the doses expressed as mean daily mg test substance/kg body weight during the gestation and lactation periods are presented in Table 3.

TABLE 3. Mean maternal test substance intake (mean mg/kg body weight/day ±SE). [a]

Period	Dose (ppm)		
	3	10	15
Gestation			
GD 0-6	0.2±0.01	0.7±0.02	1.1±0.02
GD 6-13	0.2±0.01	0.9±0.03	1.2±0.02
GD 13-20	0.2±0.00	0.7±0.01	1.1±0.03
Lactation			
LD 0-7	0.4±0.03	1.4±0.08	2.0±0.09
LD 7-14	0.5±0.01	1.9±0.06	2.9±0.06
LD 14-21	0.6±0.01	2.1±0.05	3.2±0.08

a Data obtained from Table 8, pages 73 through 75 in the study report.

4. **Reproductive performance:** Reproductive performance appeared to be unaffected by the test substance (Table 4).

TABLE 4. Reproductive performance. [a]

Observation	Dose (ppm)			
	0	3	10	15
Number mated	30	30	30	30
Number of litters	22	22	23	20
Intercurrent deaths	0	0	0	0
Mean (±SE) gestation duration (days)	21.7±0.10	21.8±0.11	21.8±0.10	21.6±0.11
Mating index (%)	100.0	100.0	100.0	100.0
Fertility index (%)	93.3	96.7	100.0	93.3
Gestation index (%)	78.6	75.9	76.7	71.4
Incidence of dystocia	NR	NR	NR	NR

a Data obtained from pages Table 1, page 59 in the study report.
NR Not reported

5. **FOB:** No treatment-related effects on FOB were noted.

6. **Maternal postmortem results**

1) **Cholinesterase determination:** Decreases ($p \leq 0.05$) in plasma (↓23-43%), erythrocyte (↓54-63%), and brain (↓19-48%) cholinesterase activities were noted in the 10 and 15 ppm dams (Table 5). Cholinesterase activity was not statistically significant at 3 ppm but apparent decreases of 14% for plasma and 11% for RBC were evident.

TABLE 5. Cholinesterase activity in dams on LD 21. [a]

Parameter	Dose (ppm)			
	0	3	10	15
Plasma (IU/mL)	0.76±0.23	0.65±0.18 (↓14%)ns	0.58±0.12* (↓23)	0.43±0.06* (↓43)
Erythrocyte (IU/mL)	1.12±0.28	1.00±0.28(↓11%)ns	0.42±0.12* (↓63)	0.51±0.26* (↓54)
Brain (IU/g)	12.8±0.8	13.0±0.6	10.4±1.5* (↓19)	6.6±1.6* (↓48)

a Data obtained from Tables CHE3-SUM, pages 876 and 877 in the study report; n=9-10.
* Significantly different from controls at $p \leq 0.05$. ns - not significant.

B. OFFSPRING

1. Viability and clinical signs: Litter size and viability (survival) results from pups during lactation are summarized in Table 6. No treatment-related findings were noted. No treatment-related clinical signs were noted during the pre-weaning or post-weaning periods.

TABLE 6. Litter size and viability. [a]

Observation	Dose (ppm)			
	Control	3	10	15
Total number born	247	252	272	242
Number born live [b]	245	251	272	242
Number born dead	2	1	0	1 [e]
Sex Ratio Day 0 (% ♂)[e]	59	64	70	71
Deaths Days 1-4 [cc]	1	0	2	1
Deaths Days 4-21 [de]	0	0	0	0
Litter size (mean ± SE)	11.2±0.41	11.5±0.43	11.8±0.32	12.1±0.45
Mean number of viable pups				
Birth	11	11	12	12
Day 4 [c]	11	11	12	12
Day 4 [d]	8	8	8	8
Day 21	8	8	8	8
Live birth index (mean ± SE, %)	99.4±0.43	99.7±0.28	100.0±0.00	100.0±0.00
Viability index (mean ± SE, %)	99.2±0.57	99.2±0.53	99.2±0.59	100.0±0.00
Lactation index (mean ± SE, %)	99.4±0.57	100.0±0.00	100.0±0.00	100.0±0.00

a Data obtained from Table 9, pages 76 through 78; and Appendix X, pages 286 through 290 in the study report.
b Calculated by the reviewers from data presented in Table 9, pages 76 through 78.
c Before standardization (culling).
d After standardization (culling).
e Calculated by the reviewers from individual data presented in Appendix X, pages 286 through 290. Does not include pups that were considered missing or cannibalized.

2. Body weight: No treatment-related differences in pre-weaning body weights were noted in any dose group (Table 7).

TABLE 7. Mean (±SE) pre-weaning pup body weights (g). [a]

PND	Dose (ppm)							
	0	3	10	15	0	3	10	15
	Males				Females			
0	6.0±0.09	5.9±0.10	6.0±0.07	5.8±0.09	5.6±0.08	5.6±0.10	5.6±0.08	5.6±0.07
4 [b]	9.7±0.23	9.9±0.21	9.9±0.20	9.6±0.17	9.3±0.24	9.7±0.21	9.4±0.21	9.2±0.17
4 [c]	9.7±0.24	9.9±0.22	9.9±0.19	9.5±0.17	9.3±0.24	9.7±0.21	9.4±0.20	9.2±0.18
11	23.4±0.71	25.1±0.47	24.3±0.40	24.3±0.44	22.7±0.72	24.8±0.48	23.6±0.45	23.7±0.43
17	36.8±1.03	38.8±0.58	38.0±0.52	38.2±0.70	35.5±0.99	37.9±0.46	36.9±0.47	37.0±0.67
21	45.2±1.30	48.9±0.85	48.3±0.64	46.8±0.91	43.1±1.24	47.5±0.73* (↑10)	46.6±0.73	44.9±0.89

a Data obtained from Table 12, pages 86 through 89 in the study report.
b Before standardization (culling).
c After standardization (culling).
* Statistically different from control, $p \leq 0.05$

No treatment-related differences in post-weaning body weights were noted in any group (Table 8). Sporadic increases ($p \leq 0.05$) in body weights were noted in the 3 and 10 ppm groups; however, theses increases were considered to be unrelated to treatment.

TABLE 8. Mean (±SD) post-weaning pup body weights (g). [a]

PwD [c] [M/F]	Dose (ppm)							
	0	3	10	15	0	3	10	15
	Males				Females			
8/9	74.0±10.5	77.8±6.3	75.8±7.2	76.2±7.0	73.2±9.5	78.3±6.2* (↑7)	76.4±6.4* (↑4)	75.7±5.8
29/30	208.8±21.2	211.4±16.3	202.6±17.5	204.4±16.2	152.7±11.5	159.1±10.1* (↑4)	150.9±10.8	152.6±10.1
50/51	312.1±29.1	315.1±24.4	303.3±28.5	309.9±23.7	192.5±14.6	196.2±15.3	189.7±13.6	191.7±15.0

a Data obtained from pages Table 15, pages 97 through 99.
* Statistically different from control, $p \leq 0.05$
c Pwd = post weaning day.

3. Developmental landmarks

a) Sexual maturation: Sexual maturation data are presented in Table 9. No treatment-related differences in the time to preputial separation or vaginal patency were noted. Slight increases ($p \leq 0.05$) in the time to preputial separation were noted in the 10 and 15 ppm males (↑2-4%); however, these increases were minor and considered not to be toxicologically important. Increased ($p \leq 0.05$) time to vaginal patency was noted in the 10 ppm females (↑6%); however, this increase was not dose-related.

TABLE 9. Mean (±SE) age of sexual maturation (days). [a]

Parameter	Dose (ppm)			
	0	3	10	15
N (M/F)	66/63	66/66	69/69	60/60
Preputial separation (males)	43.1±0.24	43.7±0.32	44.3±0.39*(↑2)	45.0±0.46** (↑4)
Vaginal opening (females)	33.1±0.25	33.9±0.33	35.2±0.59** (↑6)	34.2±0.45

a Data obtained from Table 14, pages 95 through 96 in the study report.
* Statistically different from control, p≤0.05
** Statistically different from control, p≤0.01

b) Physical landmarks: Physical landmarks were not evaluated.

4. Behavioral assessments

a) Functional observational battery: No treatment-related FOB effects were noted.

b) Motor activity: Total motor activity (including locomotor activity) was comparable between treated pups and controls during the preweaning and postweaning periods (Table 10). The total mean number of movements nearly tripled between PND 13 and 21 in all groups. Subsession data indicated that habituation was normal.

TABLE 10. Mean (±SD) motor activity data (total number of movements). [a]

Test Day	Dose (ppm)			
	0	3	10	15
Males				
PND 13	79±109	71±57	89±104	68±50
PND 17	263±153	204±112	239±116	210±104
PND 21	341±92	323±112	358±127	304±127
PND 60	573±128	512±122	511±108	534±123
Females				
PND 13	56±68	84±83	64±61	60±40
PND 17	270±140	238±125	201±89	184±114
PND 21	362±125	379±108	354±157	326±94
PND 60	744±184	691±157	755±178	698±204

a Data obtained from Table 19, pages 189 through 191 in the study report; n = 13-16.

c) Auditory startle reflex habituation: No treatment-related differences in auditory startle response were observed (Table 11). Peak amplitude and latency to peak data were similar between treated groups and controls. Data recorded for the five blocks indicated that habituation patterns were normal.

TABLE 11. Auditory startle reflex peak amplitude data (mean ±S.D.). [a]

Block		Dose (ppm)			
		0	3	10	15
Males					
PND 22	1	45±17	46±20	40±14	44±12
	2	44±21	51±24	39±17	47±19
	3	41±20	49±18	38±16	42±19
	4	39±22	49±16	37±17	42±16
	5	36±15	42±15	36±17	37±15
	Mean	41±18	47±17	38±14	42±14
PND 38	1	112±82	111±85	89±55	85±59
	2	93±68	107±76	74±41	71±44
	3	86±56	87±63	69±44	70±34
	4	61±31	68±55	63±38	52±30
	5	56±29	65±56	52±32	44±21
	Mean	82±48	87±64	69±36	64±33
PND 60	1	299±238	286±262	241±172	248±157
	2	231±164	292±262	256±175	254±205
	3	188±146	199±175	210±165	172±143
	4	175±127	171±178	156±149	150±111
	5	149±142	148±162	152±121	128±81
	Mean	208±156	219±200	203±144	190±126

(table continues next page)

Block		Dose (ppm)			
		0	3	10	15
Females					
PND 22	1	39±19	45±18	40±19	46±28
	2	41±19	46±21	46±25	45±31
	3	38±19	41±21	42±24	40±28
	4	36±18	36±17	36±20	36±23
	5	33±17	36±16	31±18	33±20
	Mean	37±17	41±17	39±20	40±25
PND 38	1	96±75	74±51	61±36	76±49
	2	85±71	67±51	60±42	57±42
	3	75±57	69±62	57±48	56±49
	4	53±36	62±51	47±40	51±43
	5	47±20	39±27	36±21	40±29
	Mean	71±47	62±42	52±35	56±40
PND 60	1	141±94	165±110	122±105	131±99
	2	149±123	130±85	126±121	121±98
	3	117±92	98±71	104±118	112±74
	4	96±64	80±47	88±94	76±61
	5	80±51	74±38	83±112	71±40
	Mean	117±79	109±60	105±106	103±70

a Data obtained from Tables 23 and 24, pages 213 through 222 in the study report; n = 14-16.

d) <u>Learning and memory testing</u>: No treatment-related differences in the passive avoidance (Table 12, day s 22 and 29) or watermaze)Table 13, days 60±2) tests were observed.

TABLE 12. Passive avoidance performance (mean ± S.D.). [a]

Session/Parameter		Dose (ppm)			
		0	3	10	15
Males					
Session 1	Trials to criterion	3.3±0.6	3.3±0.7	3.6±0.9	3.7±1.1
	Latency trial 1 (sec)	24.1±21.6	33.6±44.5	22.3±20.3	19.2±13.0
	Latency trial 2 (sec)	156.0±48.8	159.3±43.1	142.3±56.5	153.4±44.7
	Failed to learn	0	0	0	0
Session 2	Trials to criterion	2.2±0.4	2.4±0.7	2.0±0.0	2.3±0.8
	Latency trial 1 (sec)	167.6±28.1	172.7±21.7	180.0±0.0	160.2±50.3
	Latency trial 2 (sec)	180.0±0.0	173.2±24.6	180.0±0.0	180.0±0.0
Females					
Session 1	Trials to criterion	3.3±0.6	3.4±1.0	3.5±0.9	3.4±0.6
	Latency trial 1 (sec)	34.3±32.7	27.8±34.2	30.8±30.8	23.4±21.1
	Latency trial 2 (sec)	168.4±41.4	156.8±50.7	154.3±54.8	159.0±40.2
	Failed to learn	0	0	0	0
Session 2	Trials to criterion	2.4±0.7	2.6±0.9	2.8±1.1	2.4±0.6
	Latency trial 1 (sec)	168.5±31.2	137.2±60.4	144.2±54.5	163.3±31.1
	Latency trial 2 (sec)	172.3±24.3	177.8±8.7	174.7±21.1	176.8±12.9

a Data extracted from Table 25, pages 223 through 225 of the study report; n= 15-16.

TABLE 13. Water maze performance (mean ± S.D.). [a]

Session/Parameter		Dose (ppm)			
		0	3	10	15
Males					
Session 1	Trials to criterion	7.9±2.5	9.3±3.3	8.9±3.2	9.6±3.2
	Trial 1 - Errors	1.0±1.4	0.7±0.7	0.9±0.7	1.4±1.3
	Trial 1 - Duration (sec)	17.9±14.4	17.4±7.9	24.6±18.9	25.8±19.4
	Trial 2 - Errors	0.8±0.9	0.9±1.1	0.8±1.1	0.8±1.0
	Trial 2 - Duration (sec)	16.4±13.8	21.8±15.7	21.4±21.1	22.3±17.2
	Failed to meet criterion	0	0	1	2
Session 2	Trials to criterion	5.6±0.9	6.1±2.2	5.7±1.1	5.2±0.6
	Trial 1 - Errors	0.6±1.0	0.3±0.6	0.5±0.9	0.1±0.5
	Trial 1 - Duration (sec)	10.2±8.2	7.9±5.7	9.9±10.2	5.8±4.0
	Trial 2 - Errors	0.1±0.5	0.3±0.4	0.1±0.3	0.1±0.3
	Trial 2 - Duration (sec)	4.8±3.1	5.6±4.2	3.8±1.7	4.4±2.7
Females					
Session 1	Trials to criterion	7.6±3.1	8.9±3.6	7.0±2.8	7.0±2.3
	Trial 1 - Errors	0.9±1.1	0.9±0.8	0.8±1.0	0.6±1.0
	Trial 1 - Duration (sec)	16.6±12.5	14.9±13.6	15.6±9.7	13.2±8.5
	Trial 2 - Errors	0.6±1.0	0.6±0.8	0.8±1.1	1.1±1.4
	Trial 2 - Duration (sec)	10.4±8.5	12.4±9.4	13.8±14.0	16.6±14.9
	Failed to meet criterion	0	1	1	0
Session 2	Trials to criterion	7.2±3.1	5.7±1.5	6.4±2.5	5.9±1.8
	Trial 1 - Errors	0.3±0.6	0.7±1.0	0.3±0.6	0.3±0.9
	Trial 1 - Duration (sec)	6.0±3.4	11.1±8.0	7.4±6.9	6.1±6.4
	Trial 2 - Errors	0.4±0.8	0.0±0.0	0.1±0.5	0.0±0.0
	Trial 2 - Duration (sec)	5.6±5.9	4.1±2.9	4.5±3.9	3.1±1.1

a Data obtained from Table 26, pages 226 through 228 in the study report; n = 14-16.

5. Postmortem results

a) Brain weights: Absolute and relative (to body weight) brain weights were comparable between treated animals and controls on PND 21 and at study termination (Table 14).

TABLE 14. Mean (±SD) brain weights of perfused animals. [a]

Parameter	Dose (ppm)			
	0	3	10	15
Males				
PND 21				
Terminal body weight (g)	45.2±7.5	49.3±4.8	48.2±4.1	47.2±4.6
Brain weight (g)	1.411±0.075	1.397±0.053	1.422±0.049	1.426±0.079
Brain-to-body weight ratio (%)	3.203±0.571	2.864±0.341	2.965±0.208	3.049±0.344
Termination				
Terminal body weight (g)	319.5±23.2	323.7±22.1	294.4±43.3	315.6±22.6
Brain weight (g)	1.923±0.048	1.893±0.067	1.896±0.046	1.914±0.067
Brain-to-body weight ratio (%)	0.604±0.034	0.586±0.032	0.662±0.140	0.609±0.045
Females				
PND 21				
Terminal body weight (g)	42.9±4.7	46.3±3.9	46.5±2.1	44.9±4.7
Brain weight (g)	1.352±0.051	1.362±0.036	1.374±0.048	1.352±0.074
Brain-to-body weight ratio (%)	3.176±0.282	2.959±0.255	2.956±0.136	3.046±0.417
Termination				
Terminal body weight (g)	199.8±15.9	198.9±24.6	193.2±9.2	195.0±11.6
Brain weight (g)	1.765±0.105	1.794±0.067	1.734±0.068	1.768±0.047
Brain-to-body weight ratio (%)	0.886±0.055	0.912±0.089	0.900±0.054	0.909±0.043

a Data obtained from Tables OW1K-SUM and OW2K-SUM, pages 907 through 912 in the study report; n =10.

b) Neuropathology

1) Cholinesterase determination: Plasma, erythrocyte, and brain cholinesterase activities were comparable between control and treated pups on PND 4 and PND 21 (Table 15).

TABLE 15. Cholinesterase activity. [a]

Parameter	Dose (ppm)			
	0	3	10	15
PND 4 (sexes combined)				
Plasma (IU/mL)	0.63±0.08	0.65±0.07	0.62±0.05	0.64±0.09
Erythrocyte (IU/mL)	1.21±0.28	1.26±0.28	1.25±0.31	1.29±0.26
Brain (IU/g)	4.2±0.4	4.2±0.3	4.3±0.3	4.2±0.3
PND 21				
Males				
Plasma (IU/mL)	0.61±0.08	0.63±0.07	0.63±0.07	0.59±0.10
Erythrocyte (IU/mL)	1.34±0.23	1.55±0.61	1.40±0.30	1.34±0.30
Brain (IU/g)	11.6±0.6	11.2±0.5	11.4±0.5	11.1±0.4
Females				
Plasma (IU/mL)	0.53±0.08	0.55±0.08	0.58±0.11	0.60±0.08
Erythrocyte (IU/mL)	1.30±0.35	1.17±0.20	1.19±0.32	1.15±0.18
Brain (IU/g)	11.4±0.5	11.6±0.04	11.6±0.5	11.4±0.3

a Data obtained from Tables CHE1-SUM and CHE2-SUM, pages 871 through 875 in the study report; n= 18-21 for PND 4; n=5-10 for PND 21.

2) **Macroscopic examination:** No treatment-related findings were noted.

3) **Microscopic examination:** No treatment-related findings were noted.

4) **Morphometric evaluation:** All morphometric measurements were similar between treated animals and controls (Table 16).

TABLE 16. Morphometric measurements. [a]

Parameter (μm)	Dose (ppm)			
	0	3	10	15
Males				
PND 21				
Frontal cortex	1.8002±0.07	NA	NA	1.7553±0.11
Caudate putamen	3.2160±0.32	NA	NA	3.0893±0.21
Parietal cortex	1.8928±0.11	NA	NA	1.8712±0.12
Corpus callosum	0.3627±0.04	NA	NA	0.3916±0.10
Hippocampal gyrus	1.6278±0.13	NA	NA	1.6405±0.15
Cerebellum	4.5632±0.42	NA	NA	4.5149±0.33
Termination				
Frontal cortex	1.8771±0.11	NA	NA	1.8429±0.07
Caudate putamen	3.4702±0.11	NA	NA	3.4539±0.14
Parietal cortex	1.8894±0.06	NA	NA	1.8639±0.09
Corpus callosum	0.5441±0.10	NA	NA	0.5092±0.06
Hippocampal gyrus	1.6472±0.59	NA	NA	1.6311±0.58
Cerebellum	3.9098±1.38	NA	NA	4.6227±0.40
Females				
PND 21				
Frontal cortex	1.7580±0.06	NA	NA	1.8079±0.11
Caudate putamen	3.1075±0.13	NA	NA	3.1797±0.14
Parietal cortex	1.8372±0.09	NA	NA	1.8425±0.08
Corpus callosum	0.4190±0.14	NA	NA	0.4473±0.09
Hippocampal gyrus	1.6164±0.14	NA	NA	1.5372±0.13
Cerebellum	4.3913±0.20	NA	NA	4.3360±0.16
Termination				
Frontal cortex	1.7795±0.16	NA	NA	1.7062±0.11
Caudate putamen	3.4099±0.19	NA	NA	3.3442±0.09
Parietal cortex	1.7682±0.13	NA	NA	1.7171±0.11
Corpus callosum	0.5525±0.04	NA	NA	0.5347±0.04
Hippocampal gyrus	1.4840±0.57	NA	NA	1.5729±0.16
Cerebellum	4.3276±0.35	NA	NA	4.2045±0.23

a Data obtained from Tables BM1-SUM and BM2-SUM, pages 916 through 925 in the study report; n= 9-10.
NA Not applicable

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Administration of azinphos-methyl in the diet resulted in decreased maternal plasma, erythrocyte, and brain cholinesterase activities at 10 and 15 ppm. No systemic toxicity was observed in the maternal animals. No treatment-related findings were noted in the offspring. No evidence of developmental neurotoxicity was observed at any dose tested. The maternal NOAEL was 3 ppm based on decreased cholinesterase. The offspring NOAEL was 15 ppm.

B. REVIEWER COMMENTS

1. Parental animals: No treatment-related findings, with the exception of cholinesterase inhibition, were noted at any dose tested.

The maternal LOAEL was not observed. The maternal NOAEL is 15 ppm.

2. Offspring: No treatment-related findings were noted at any dose tested.

The offspring LOAEL was not observed. The offspring NOAEL is 15 ppm.

3. Cholinesterase: Decreases ($p \leq 0.05$) in plasma (↓23-43%), erythrocyte (↓54-63%), and brain (↓19-48%) cholinesterase activities were noted in the 10 and 15 ppm dams. Cholinesterase activity was unaffected by treatment at 3 ppm. Plasma, erythrocyte, and brain cholinesterase activities were comparable between control and treated pups on PND 4 and PND 21.

The plasma, erythrocyte, and brain cholinesterase LOAEL is 10 ppm based on cholinesterase inhibition in the dams. The plasma, erythrocyte, and brain cholinesterase NOAEL is 3 ppm.

There was no evidence of developmental neurotoxicity.

This study is classified **UNACCEPTABLE/Guideline** and does not satisfy the guideline requirement (OPPTS 870.6300; OECD 426) for a developmental neurotoxicity study in rats. It is noted that neither maternal nor offspring systemic toxicity were observed and the limiting factor for not accepting this study is that there is no evidence that the pups were actually exposed to azinphos methyl. Although the results from one-generation and two-generation studies indicated that there os a steep dose response and there is some support for the selection of the dose levels used, there is still no proof that the pups were actually exposed to the test material. The purpose of a developmental neurotoxicity study is to assess whether or not exposure to the pups during gestation and in lactation can result in developmental effects. Since there was no evidence that the pups were actually exposed through lactation, the study is not acceptable in terms of current criteria for acceptance of a developmental toxicity study.

C. STUDY DEFICIENCIES: The following deficiencies were noted, but do not affect the results of this study:

- Physical landmarks (incisor eruption, eye opening) were not evaluated.
- No positive control data were provided with the study report. Note added by the HED reviewer: HED has on file positive control studies from the Beyer laboratory.

DATA FOR ENTRY INTO ISIS

Developmental Neurotoxicity Study - rats (870.6300)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
058001	45711201	dev neurotox	rats	GD 0-LD 21	oral	diet	3-15	0, 3, 10, 15	15	Not observed		Maternal, dose in ppm
058001	45711201	dev neurotox	rats	GD 0-LD 21	oral	diet	3-15	0, 3, 10, 15	15	Not observed		Offspring, dose in ppm
058001	45711201	dev neurotox	rats	GD 0-LD 21	oral	diet	3-15	0, 3, 10, 15	3	10	maternal plasma, erythrocyte, and brain ChE inhibition	Cholinesterase, dose in ppm

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Chemical:	**Azinphos-Methyl**
PC Code:	**058001**
HED File Code	**13000 Tox Reviews**
Memo Date:	**06/28/2004**
File ID:	**TX0050916**
Accession Number:	**412-05-1000**

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09/07/2004